was then placed in a homogenizer tube and 0.05 cc of a 10% solution of the drug in peanut oil per gram of brain was added. Pure oil was added to controls. The brain and the oil solutions were then homogenized. Sufficient quantity of a modified Krebs phosphate solution (containing 0.1% glucose) was added to make the final homogenate, containing 125 mg brain (wet weight) in 3 cc. This technique was necessitated by the low water solubility of the BHC isomers. The homogenate was equilibrated with room air at 38° C for 10 min and allowed an additional equilibration period of 10 min after the vessels were closed. Readings were taken at 15-min intervals for a period of 1 hr.

TABLE 1

EFFECT OF ISOMERS OF BENZENE HEXACHLORIDE ON BRAIN OXYGEN UPTAKE

Compound	Brain (mg/gm)	No. of determinations	QO_2 (wet wt)	Ratio experimental/control
Control	....	9	0.79 ± 0.1*	1.00
Alpha	2.5	6	0.92 ± 0.07	1.16
	5.0	6	0.97 ± 0.07	1.23
Gamma	5.0	11	0.76 ± 0.06	0.96
Delta	0.5	4	0.93 ± 0.10	1.18
	1.25	5	1.13 ± 0.09	1.43
	2.5	5	1.23 ± 0.13	1.56
	3.75	5	1.46 ± 0.07	1.85
	5.0	11	1.40 ± 0.16	1.77
Delta + Gamma	5.0 + 5.0	4	1.41 ± 0.19	1.78
Delta + Inositol	5.0 + 25.0	1	1.35	1.71
Gamma + Inositol	5.0 + 25.0	1	0.77	0.97

* $\sqrt{\frac{\Sigma d^2}{N}}$

The QO_2 values obtained on rat brain homogenates containing added drugs are shown in Table 1. Whereas γ-BHC failed to alter the oxygen uptake of the brain, the delta isomer significantly increased this function. Inositol did not alter the oxygen uptake produced by either isomer and γ-BHC exhibited no antagonism against the effects of the delta isomer.

The most striking fact obtained from this study was the unexpected position of the two isomers with respect to their effects upon the brain-homogenate respiration. The narcotic isomer (delta) produced as high as an 80% increase in respiration over the control. Moreover, its pharmacological antagonist, the gamma isomer, a powerful convulsant, did not modify this effect. In addition, *i*-inositol was similarly without effect. The "stimulating effect" of the delta isomer upon the respiration of brain homogenate in the face of its narcotic properties *in vivo* is not yet understood and requires further study. The possibility that this increased oxygen consumption is merely a reflection of the oxidation of the halogenated hydrocarbon cannot be overlooked. However, it is interesting to consider these facts in light of the previously mentioned hypothesis of the mechanism of action of certain narcotic agents.

The unaltered QO_2 values observed in the exploratory studies on rats poisoned prior to decapitation is also noteworthy. Similar results have often been observed for other anesthetics which depress the oxygen uptake of the brain on direct addition *in vitro*. Quastel (7) explains this by an outward diffusion of the drug from the cells to the medium. It does not seem likely that this explanation would hold in the case of the isomers of BHC since these compounds are oil rather than water soluble. No explanation for this divergence can be offered at this time.

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Color Change of Strawberry Anthocyanin with D-Glucose[1]

Ernest Sondheimer and Frank A. Lee

Division of Food Science and Technology,
New York State Agricultural Experiment Station,
Cornell University, Geneva

Strawberries (garden varieties) are usually prepared by mixing with granulated sucrose prior to freezing. Due to the scarcity of this sugar during the war years, experiments to substitute other sweetening agents were initiated. When crystalline glucose was used a violet-to-blue coloration developed on freezing, which reverted to the original red color on thawing. This color change with glucose may be peculiar to strawberries, since it has not been observed with small fruits such as red raspberries. Interest in the applied as well as in the fundamental aspects of this phenomenon led us to an investigation.

Customarily, sliced strawberries are frozen with sucrose in a ratio of four parts of fruit to one part of sugar. In this preparation no change in color occurs. However, thirty varieties of strawberries grown in Geneva all turned violet when frozen with ordinary commercial crystalline glucose at −23.3° C. in a four to one

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ratio. Differences in the time required to produce the color change were observed. Ten samples of the same variety (Dresden) showed color variations of essentially the same order of magnitude as those obtained with the different varieties. The only exception was a recently named strawberry, Fairland, which showed violet color only after 27 days' storage. Common varieties like Marshall, Blakemore, Dresden, Howard 17 and Culver all turned violet in one to five days. The minimum amount of glucose required to produce the violet color in the strawberries was found to depend largely on the presence of undissolved glucose. Violet color formation was observed only in those samples which contained glucose in a solid form. It is significant to note that all the violet material was always found associated with the undissolved glucose and that in those places where there was no solid glucose the strawberry preparation stayed red. Whether the glucose crystallized during storage or whether it was originally present as a solid did not seem to affect the color change. Another observation was that the time of separation of the glucose often does not coincide with the first appearance of the violet color.

As was expected, the anthocyanin pigment of strawberries plays a dominant role in the violet color development. When the anthocyanin chloride (*1*) (believed to be mainly pelargonidin 3-monoglucoside) is dissolved in Sorensen's sodium citrate-hydrochloric acid buffer of pH 3.4, or distilled water, and the solution is saturated with chemically pure D-glucose, and frozen at −23.3° C, a violet color is obtained. If similar solutions are allowed to evaporate at room temperature, the anthocyanin also turns violet. This residue is stable at room temperatures. The anthocyanin reverts to the red form when the frozen samples are thawed or when distilled water is added to the dry material.

These tests led to the conclusions that (1) the violet color is not due to possible impurities of the glucose used since c.p. D-glucose gives identical results; (2) low temperatures are not necessary for the formation of the violet color; and (3) the color change is reversible. The rate of color formation is influenced by the pigment concentration. Solutions containing 25% glucose showed violet spots after 10 days' storage at −23.3° C only if the anthocyanin concentration was 12 mg% or higher. The role of pH seems to be of minor importance. When the pH of frozen samples is lowered to pH 3.0, 2.5 and 1.8 a retarding affect with increasing acidity is noted. The air-dried, violet anthocyanin-glucose mixture turns red on heating. The transition temperature, which is not very sharp, is between 65° and 70° C for the pulverized material. Whether or not a loss of water occurred during the heating period was not determined.

Although a number of sugars were tested, only glucose gave a characteristic color change with the anthocyanin. Tests with sucrose, galactose, rhamnose, fructose, maltose, and α methyl glucoside were negative. Several of these sugars did not separate from solution under the conditions of the experiment and this may have caused the negative results. Inconclusive results were obtained with invert sugar. Arabinose seems to have a very slight effect.

Should it be found that the described reaction really is specific for pelargonidin 3-monoglucoside (or other glycosides of pelargonidin) it could well serve as a qualitative test for these compounds.

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The Effect of Pteroylglutamic Acid on the Aromatic Amino Acid Metabolism of Premature Infants[1]

C. D. Govan, Jr.,[2] and Harry H. Gordon

Departments of Pediatrics and Biochemistry, University of Colorado Medical Center, Denver

Scorbutic guinea pigs fed tyrosine (*4*) and prematurely born infants fed cow's milk mixtures of relatively high protein content (*3*) excrete large amounts of hydroxyphenyl derivatives in the urine. These can be decreased markedly by the administration of ascorbic acid (*3*, *4*). Woodruff and Darby (*5*) studied the effect of administration of PGA on the excretion of hydroxyphenyl derivatives by scorbutic guinea pigs and found that PGA repaired the defect produced by lack of ascorbic acid. Johnson and Dana (*2*) have reported that ascorbic acid produced significant increases in weight gain and leucocyte and normoblast count in PGA-deficient rats.

TABLE 1

EFFECT OF ORAL PGA ON "TYROSYL" EXCRETION

Infant B. L.—weight at birth: 2.15 kg			
Age in days	Weight in kg	Urine "tyrosyl"	
		mg/cc	mg/kg/24 hr
14	2.04	1.5	40
16	2.05	1.1	34
21	2.47	.4	18
29	3.98	5.2	289
30	2.95	5.4	357
31	PGA—5 mg started daily by gavage		
34	2.95	4.8	368
37	3.09	4.3	279
41	3.10	3.4	234
42	PGA—10 mg started daily by gavage		
44	3.26	3.1	267
46	3.40	1.4	112
50	3.52	0.2	17

In the present study, PGA was given to 10 premature infants fed cow's milk mixtures containing 6 g of protein and 120 cal per kg of body weight per day, but no ascorbic acid. Urine was collected for timed periods

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